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Additional DNA barcodes confirm recent morphological species concepts and synonymies in *Callomyia* Meigen (Diptera: Platypezidae)

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Cumming & Wheeler (2016) revised the Nearctic species of the sexually dimorphic flat-footed fly genus *Callomyia* Meigen (Callomyiinae) recognizing 10 Nearctic species, including three newly described taxa, namely *C. argentea* Cumming, *C. arnaudi* Cumming, and *C. browni* Cumming. They also described the unknown female of *C. velutina* Johnson and proposed three new synonymies associating species previously described from one sex only with others described from the opposite sex (Kessel 1948; Kessel & Buegler 1972). *Callomyia cleta* Kessel was considered a junior synonym of *C. calla* Kessel, *C. clara* Kessel was considered a junior synonym of *C. corvina* Kessel, and *C. liardia* Kessel & Buegler was synonymized with *C. proxima* Johnson.

These taxonomic decisions were made primarily on the basis of morphological data. They were corroborated wherever possible with molecular sequence data (DNA barcodes) from 658 base pairs (bp) from the 5'-end of the Cytochrome *c* Oxidase I (COI) mitochondrial gene. However, these sequences were difficult to obtain as only 17 specimens out of 26 submitted, yielded successful barcodes at that time (Cumming & Wheeler 2016, fig. 78). Recently, we have obtained new barcode sequences from 13 additional *Callomyia* specimens. These new barcodes, in combination with those published by Cumming & Wheeler (2016), now appear to support their previous taxonomic decisions.

Some of the new barcode sequences were obtained from additional fresh specimens collected after Cumming & Wheeler's (2016) revision, using the protocols outlined in that study. However, a number of barcodes were obtained from older specimens that were re-sequenced by the Biodiversity Institute of Ontario in Guelph, ON, Canada using the new technique of single molecule real-time (SMRT) sequencing as described by Herbert et al. (2018). These specimens each produced full 658 bp sequences, which were analyzed together with the previously published sequences using a Neighbour-Joining tree created in the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert 2007). COI sequences that clustered together with <2% genetic divergence in the Neighbour-Joining tree were considered to belong to the same species (Hebert et al. 2003). Barcode sequences and additional data for all the specimens are available through the Barcode of Life Data System (http://www.barcodinglife.org/). The 13 new COI sequences were also deposited in GenBank, indicated in Figure 1 with GenBank numbers beginning with the prefix MN555.

The new Neighbour-Joining tree is shown in Figure 1. Thirty *Callomyia* specimens are included in the tree representing 10 species, as well as two additional Callomyiinae outgroup species used to root the tree. Seven Nearctic species are now included in the tree, as well as three Palaearctic species, namely *C. amoena* Meigen, *C. dives* Zetterstedt and *C. speciosa* Meigen. Even with the inclusion of additional specimens and species in the new Neighbour-Joining tree, no Holarctic species were revealed, which agrees with the previous conclusions of Cumming & Wheeler (2016). Two of their newly described species, namely *C. argentea* and *C. arnaudi* are now supported as distinct species by the barcode sequence data. Their third new species, *C. browni*, is still only known from a single specimen and has not yet been barcoded. *Callomyia corvina* described by Kessel (1948) from males only is also depicted as a distinct species in the Neighbour-Joining tree, but the female described in the same paper by Kessel as *C. clara* has yet to be barcoded, so Cumming & Wheeler's (2016) synonymy of these two species has not been verified molecularly. However, their synonymy of *C. cleta* under *C. calla* is now confirmed with the new barcode sequence data, and their previous synonymy of *C. liardia* under *C. proxima*, using both morphology and barcode data, is strengthened with the inclusion of another barcoded specimen (CNC287574). One male specimen (DEBU 00075059), which is morphologically identical to *C. liardia* (= *C. proxima*), continues to yield a barcode with >2% genetic divergence from the remainder of the *C. proxima/ C. liardia* cluster. As previously suggested

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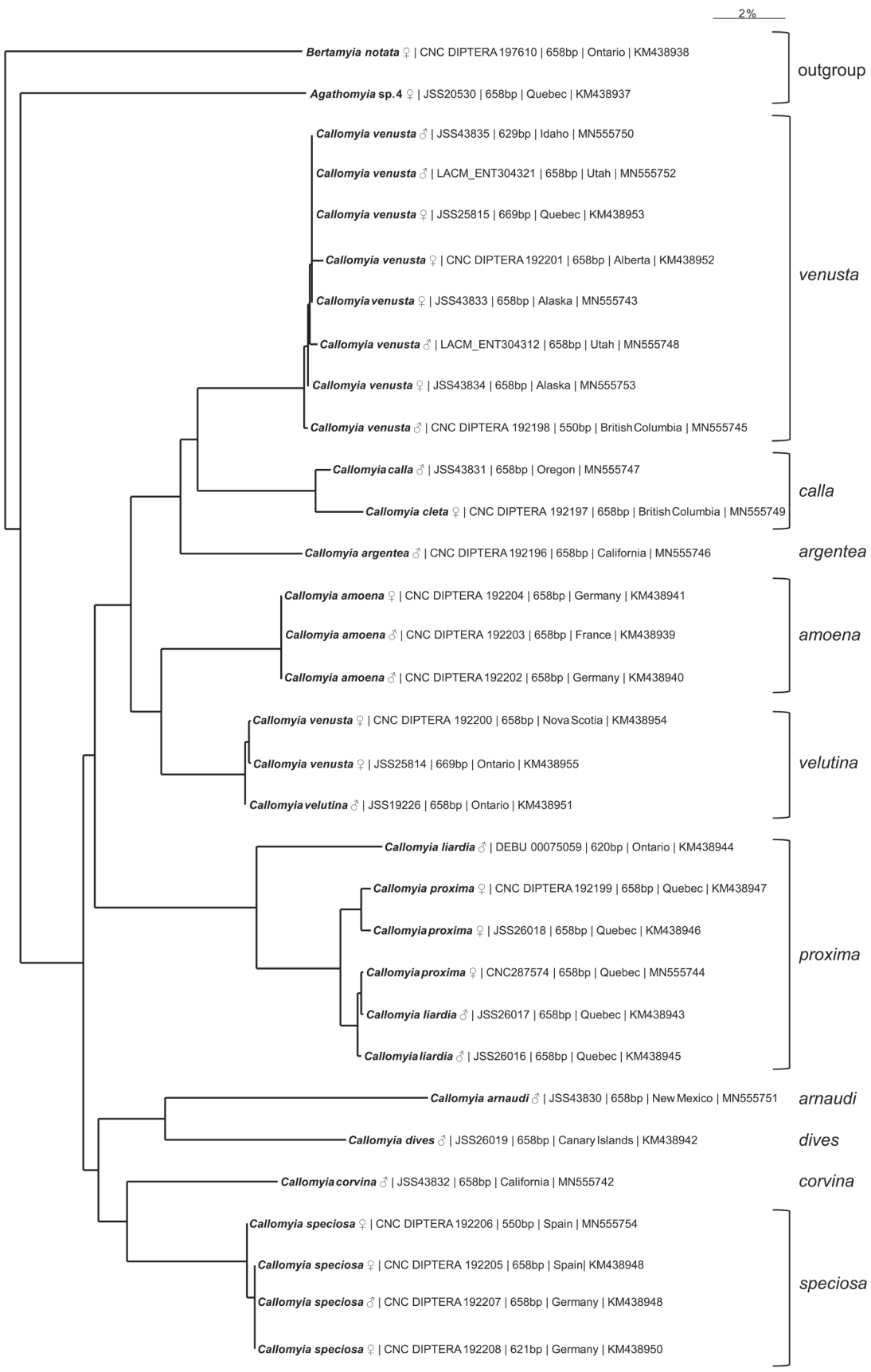

FIGURE 1. Neighbour-Joining tree of 30 *Callomyia* specimens and two outgroup specimens with COI sequences ≥550 bp, including sex, unique voucher number, sequence length, geographic locality and GenBank number. Bracketed species names are the current species concepts used in Cumming & Wheeler (2016) and include Nearctic synonyms and misidentifications (shown on branch terminals) that are found in Kessel & Buegler (1972).

by Cumming & Wheeler (2016), the shorter sequence obtained from that specimen (only 620 bp compared with 658 bp for the other five) might explain its higher 6% genetic divergence from the rest of the *C. proxima* cluster, although other factors such as initial specimen preservation could be involved. Finally, four new male and two new female specimens of *C. venusta* Snow are included on the Neighbour-Joining tree, in addition to the two female specimens of *C. venusta* originally barcoded by Cumming & Wheeler (2016). This supplemental information is important, because it confirms some of the abdominal colour variation they reported for *C. venusta* and indicates that the disjunct eastern and western populations of this species are conspecific. It also supports their recognition and description of the cryptic female of *C. velutina*, which was previously unknown and confused with females of *C. venusta*.

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